



## **Survey of Specific Fish Pathogens in Free-ranging Fish from Devils Lake and the Sheyenne and Red Rivers in North Dakota.**



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Devils Lake and the Sheyenne and Red Rivers in North Dakota.**

**In cooperation with**

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## Executive Summary

During October 2001 and August 2002, more than 500 fish were collected from Devils Lake, the Sheyenne River, and the Red River and tested for a list of specific fish pathogens using protocols for the U. S. Fish and Wildlife Service National Wild Fish Health Survey. The survey was performed to establish baseline data in response to concerns for the transfer of biota from either natural overflow or from construction of an outlet that would connect Devils Lake to the Sheyenne and Red rivers. Testing for fish pathogens involved three main components. First, fish were examined externally and internally for gross signs of disease or abnormalities. Next, specific tissues were collected aseptically and screened for pathogens using standardized assays. Lastly, tissues were subjected to further testing with highly specific corroborative or confirmatory assays whenever suspect pathogens were detected with screening methods. Results of the survey are presented in this report and can also be viewed graphically on the world wide web by logging onto the National Wild Fish Health Survey Database: <http://wildfishsurvey.fws.gov>.

At Devils Lake, 180 fish were collected over two consecutive days of sampling. Nets and traps were set in a northeastern area of the lake known as Six Mile Bay. The catch was composed of walleye, northern pike, black crappie, and yellow perch. Antigen of *Renibacterium salmoninarum*, causative agent of bacterial kidney disease (BKD), was detected in very low levels from northern pike, walleye, and yellow perch. Active infection could not be confirmed in these populations using a highly specific DNA-based polymerase chain reaction (PCR) assay. Overall, fish appeared in good general condition. We did not observe any external or internal signs of BKD or any other disease or abnormality. No other listed fish pathogens were detected.

A total of 275 fish, representing ten species, were collected among two sampling sites on the Sheyenne River. Catch rates on the Red River were lower than Devils Lake and the Sheyenne River. A total of 83 fish, representing eight species, were collected from two sampling sites. Antigen of *R. salmoninarum* was detected in low or medium levels in all species although antigen was not found in northern pike, smallmouth bass, walleye, and white sucker from the downstream site near Valley City. Nearly 40 kidney samples from fish collected in the Red and Sheyenne rivers were tested with the PCR and all were negative for *R. salmoninarum*. No clinical signs of BKD or other diseases or abnormalities were observed, and no other listed fish pathogens were detected.

It may be that low levels of antigen detected with the *R. salmoninarum* ELISA used for screening represent false positive readings or background noise. Many of the ELISA OD values were just slightly above the negative-positive threshold. This threshold value is established each time the assay is performed using a standardized reference tissue from chinook salmon. Some have argued that reference tissue used to calculate a negative-positive threshold be established for each family of fish tested.

In summary, fish from across the survey area appeared in be in good health and condition. We did not observe any external or internal signs that would indicate fish were affected by

disease. Many fish screened were suspected positive for *R. salmoninarum* but results of those tests could not be confirmed with the highly sensitive and specific PCR assay. None of the other specific fish pathogens listed in the survey were detected from Devils Lake or the Red and Sheyenne rivers.

### **Acknowledgments**

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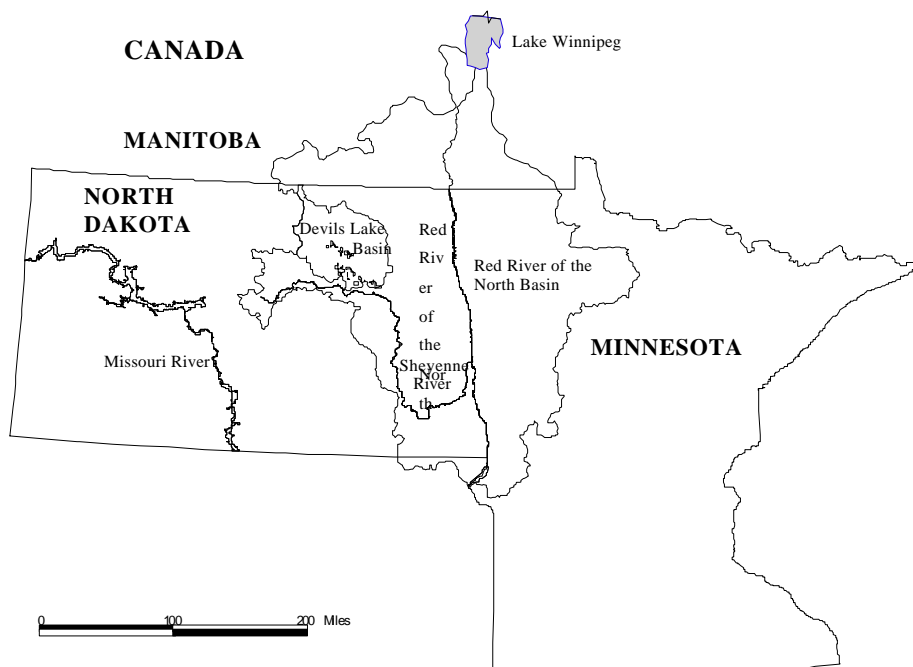
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## Introduction

At the request of the U. S. Army Corps of Engineers, the Bozeman Fish Health Center performed a fish pathogen survey at Devils Lake, the Sheyenne River, and the Red River of the North in North Dakota. The survey was conducted in response to concerns for biota transfer from either natural overflow or construction of an outlet that would connect Devils Lake to the Sheyenne and Red rivers within the Hudson Bay drainage (Figure 1).

Devils Lake is essentially a closed basin with no perennial outlet and levels are affected primarily by rainfall, runoff, and evapotranspiration. Water levels in the lake have fluctuated for thousands of years. The lake was at its lowest level during the 1940's and has been on the rise since then with significant increases during the last decade. Devils Lake would spill naturally into the Sheyenne River at an elevation of about 1459 ft above mean sea-level. Its current level of approximately 1447 ft is about 12 ft below the point of overflow. Devils Lake is believed to have overflowed into the Sheyenne River at least once in the last 1200 years. Because the lake has been isolated (closed) for many years, there is concern for the effects it may have on the Sheyenne and Red rivers in the event of spillover. Likewise, there is considerable controversy associated with construction of an outlet including water quality, biota transfer, social and economic, erosion, induced flooding, effectiveness, and reduced probability of natural spill, among others.



**Figure 1: Location of Devils Lake basin**  
**(Source: Integrated Planning Report and**  
**Environmental Impact Statement, USACE, 2002)**

The goal of this study was to collect representative fish from Devils Lake, Sheyenne River, and Red River and test their tissues for specific fish pathogens under guidelines of the National Wild Fish Health Survey (NWFHS; U. S. Fish & Wildlife Service 2001). In this report we address primarily the bacterial and viral fish pathogen component of the NWFHS and its application to the biota transfer issue (Table 1). Little historical information on the distribution and prevalence of specific bacterial and viral fish pathogens is available for the study area and most work has focused on surveys for fish parasites (Forstie and Holloway 1984; Reinisch 1981; Sutherland and Holloway 1979). Between 1993 and 1997, Minnesota Department of Natural Resources collected approximately 140 fish from the Red River and performed tests for bacteria and channel catfish virus. No pathogens of concern were detected in those surveys (J. Marcino, pers. comm.).

## Methods

Fish from Devils Lake were sampled during October 2001. The Red and Sheyenne rivers were sampled in August 2002. Fish were collected by the U. S. Fish & Wildlife Service, Missouri River Fish and Wildlife Management Assistance Office, using a variety of gear types including variegated gill nets and modified fyke nets. Nets and traps were generally set overnight for approximately 12 - 18 h durations. We established a target sample size of 60 fish for each species of interest in each body of water. At Devils Lake, nets and traps were set in Six Mile Bay for two consecutive days. We selected two sample sites on each river and designated the sites as upstream and downstream in relation to each other. River sampling sites were fished one day each. On the Sheyenne River, the upstream site was located where North Dakota Highway 20 crosses the river along the southeastern border of the Spirit Lake Reservation. The downstream site was located near Valley City from Chautauqua Park to Valley City National Fish Hatchery. On the Red River, the upstream sample site was located in Fargo upriver from the bridge at 52<sup>nd</sup> Avenue south. The downstream sites was in Grand Forks near the bridge on State Highway 2.



Fish were sorted by species, measured (mm) for total length (Krentz 2002), and then examined externally and internally for clinical signs of disease or other abnormalities. Tissues samples for pathogen testing were collected using aseptic techniques and packed in coolers with ice for transfer to the laboratory. Samples were assayed at Bozeman Fish Health Center, Bozeman, Montana according to protocols and procedures for the NWFHS (U. S. Fish and Wildlife Service 2001). Principle fish pathogens of the NWFHS included specific organisms



that are known to cause disease in cultured or wild fish, and are considered prohibitive organisms in most state and federal fish health inspection programs (Table 1). Additionally, many of the general screening methods used for the survey were also sensitive to other bacterial and viral fish pathogens.

Table 1. List of primary fish pathogens for the National Wild Fish Health Survey.

Type of pathogen	Pathogen and associated disease
Virus	Channel Catfish Virus, CCV disease
	Infectious Hematopoietic Necrosis Virus, IHN
	Infectious Pancreatic Necrosis Virus, IPN
	Largemouth Bass Virus, LMBV disease
	<i>Oncorhynchus masou</i> Virus, OMV disease
	Viral Hemorrhagic Septicemia Virus, VHS
Bacteria	<i>Aeromonas salmonicida</i> , furunculosis and ulcer disease
	<i>Renibacterium salmoninarum</i> , bacterial kidney disease
	<i>Yersinia ruckeri</i> , enteric redmouth disease
	<i>Edwardsiella ictaluri</i> , enteric septicemia of catfish
	<i>Edwardsiella tarda</i> , edwardsiellosis
Parasite	<i>Myxobolus cerebralis</i> , whirling disease

Standard cell culture techniques were used to test fish for viral pathogens. Samples of kidney and spleen were pooled from five or less fish. After dilution and maceration, kidney-spleen homogenates from all species of fish were inoculated onto *Epithelioma papulosum cyprini* (EPC) and chinook salmon embryo-214 (CHSE-214) cell lines and incubated at 15°C. To test for largemouth bass virus, tissue homogenates including swim bladder were also inoculated onto bluegill fry (BF-2) and fathead minnow (FHM) cell lines and incubated at 25°C. For Ictalurids (catfish), tissues were also inoculated onto FHM and brown bullhead (BB) cell lines and incubated at 25°C. Viral assays were begun within 72 h of collection and were monitored for cytopathic effect for 28 d.



Kidney tissue was streaked onto brain-heart infusion agar for general isolation of aerobic bacterial pathogens. Suspect bacterial growth was subcultured for purity and then differentiated with standard biochemical techniques. Kidney tissue was also collected to quantify soluble antigen of *R. salmoninarum* by the enzyme-linked immunosorbent assay (ELISA; Pascho and Mulcahy 1987). After collections for the preceding assays, the remaining kidney tissue of fish <150 mm total length was frequently insufficient to test these fish individually. In those cases, kidneys from two to five fish were pooled into a single sample until a suitable quantity of tissue was obtained for the ELISA. Only kidney tissue from the same species and sample site was pooled. Samples were run in replicate and results of the ELISA were reported as the mean optical density (OD). Standardized negative reference tissue was used to determine the threshold of detection of *R. salmoninarum* by the ELISA. The threshold of detection was calculated by adding the mean OD plus 2 SD of at least four negative controls. Kidney samples with mean ELISA OD values above the threshold were considered positive for soluble antigen of *R. salmoninarum* and were assigned to antigen level categories: OD values from the detection threshold to 0.199 were defined as low, 0.200 - 0.999 medium, and values of 1.00 or higher were considered high antigen levels (Pascho et al. 1991). When ever positive ELISA values were observed, we attempted to verify infection with *R. salmoninarum* in each species of fish using a nested polymerase chain reaction (PCR) assay (Pascho et al. 1998). Pelleted kidney tissue remaining from the ELISA sample was used in the PCR. Generally, three samples having the highest ELISA OD values were selected for each species per sample site. In cases where a species exhibited a broad range of positive ELISA values, we selected one sample each representing the upper, middle, and lower portions of the range. DNA template was extracted from suspect kidney tissue with a commercial kit and then amplified according to the PCR procedure. DNA was subjected to electrophoresis in a 1.5% agarose gel, and then stained with ethidium bromide and visualized with UV light.

*Myxobolus cerebralis*, the parasite responsible for whirling disease, was the single primary parasitic fish pathogen included in the NWFHS and in this study. However, *M. cerebralis* is considered an obligate pathogen of the family Salmonidae and all fish sampled in this study were non-salmonids. For this reason, we selectively sub-sampled fish at some locations simply because, to our knowledge, no prior testing had been done. To test for *Myxobolus cerebralis*, whole heads including gill arches were removed. Heads were halved along the median plane with one half used to screen for myxosporean spores and the other half archived frozen. Up to five half heads were pooled into a single sample. Only fish of the same species collected from the same sample site were pooled. Upon removal of soft tissues, cartilage and bone were processed enzymatically using the pepsin-trypsin digest method. After final centrifugation, pelleted samples were preserved in 70% ETOH and then examined with light microscopy. When suspect myxospores were observed, pelleted material from the enzymatic digest procedure was tested for *M. cerebralis* with a nested PCR assay (Andree et al. 1998). Archived heads were available for further testing with PCR or histology.

## Results

**Sampling.**— A total of 180 fish were collected from Devils Lake and processed for pathogen testing in 2001 (Table 2). Samples were collected from four species including black crappie *Pomoxis nigromaculatus*, northern pike *Esox lucius*, walleye *Stizostedion vitreum*, and yellow perch *Perca flavescens*. The target sample size of 60 fish for each species was collected for walleye and yellow perch, however fewer northern pike and black crappie were obtained. Net catches were not necessarily representative of species composition due to sampling design and windy weather.

Table 2.— Composition of fish collected from Devils Lake during October 2001.

Devils Lake sample site	Number of fish sampled			
	Black crappie	Northern pike	Walleye	Yellow perch
Nine Mile Bay	19	41	60	60

On the Sheyenne River, a total of 275 fish were collected from two sites during August 2002 (Table 3). Samples were collected from ten different species and consisted of black bullhead *Ictalurus melas*, black crappie, common carp *Cyprinus carpio*, green sunfish *Lepomis cyanellus*, northern pike, smallmouth bass *Micropterus dolomieu*, tadpole madtom *Noturus gyrinus*, walleye, white sucker *Catostomas commersoni*, and yellow perch. The target sample size of 60 fish was obtained for black bullhead at both the upstream and downstream sample sites and for black crappie at the downstream site. Poor catch rates for other species was attributed primarily to low abundance.

Table 3.— Composition of fish collected from two sites on the Sheyenne River during August 2002. Common names of fish are abbreviated BLB = black bullhead, BLC = black crappie, CAP = common carp, GSF = green sunfish, NOP = northern pike, SNB = smallmouth bass, TPM = tadpole madtom, WAE = walleye, WHS = white sucker, and YEP = yellow perch.

Sample site	Number of fish sampled									
	BLB	BLC	CAP	GSF	NOP	SMB	TPM	WAE	WHS	YEP
Upstream	60	0	0	0	7	0	15	15	20	0
Downstream	60	60	3	1	3	8	3	12	2	6
Total	120	60	3	1	10	8	18	27	22	6

On the Red River, we collected a total of 83 fish from two sampling sites during August 2002 (Table 3). Samples were collected from eight species which included black bullhead, common carp, channel catfish *Ictalurus punctatus*, drum *Aplodinotus grunniens*, mooneye *Hiodon tergisus*, northern pike, shorthead redhorse *Moxostoma macrolepidotum*, and white sucker. The target sample size was collected for channel catfish at the upstream site but catch rates were much lower for other species.

Table 4.— Composition of fish collected from two sites on the Red River during August 2002. Common name abbreviations for fish are explained in Table 2 and include CCF = channel catfish, DRM = drum, MOO = mooneye, and SHR = shorthead redhorse.

Sample site	Number of fish sampled							
	BLB	CAP	CCF	DRM	MOO	NOP	SHR	WHS
Up stream	0	2	60	2	0	0	1	1
Down stream	2	4	9	0	1	1	0	0
Total	2	6	69	2	1	1	1	1

**Bacterial Fish Pathogens.**— Primary culture tests from across the survey area were negative for *Aeromonas salmonicida*, bacterial agent of furunculosis, *Yersinia ruckeri*, causative agent of enteric redmouth disease, *Edwardsiella ictaluri*, cause of furunculosis, and *E. tarda*, cause of edwardsiellosis.

Antigen of *R. salmoninarum*, causative agent of bacterial kidney disease (BKD), was detected by ELISA in kidney tissue of northern pike, walleye, and yellow perch from Devils Lake (Table 5). Walleye had the lowest percentage of positive OD readings (18.7%) while northern pike had the highest (43.9%). The proportion of yellow perch with positive OD values was only slightly higher than walleye although only five perch samples were tested because most fish were too small to obtain sufficient kidney tissue for assay. The mean level of *R. salmoninarum* antigen was low for all species and many positive sample were just slightly above the negative threshold. We were unable to confirm active infection of *R. salmoninarum* in fish from Devils Lake. DNA of *R. salmoninarum* was not detected with PCR in any sample tested ( $n = 7$ ) regardless of ELISA OD value or host species. No fish, regardless of species or size, had any external or internal clinical signs indicative of BKD. Black crappie were not test for *R. salmoninarum* because fish were too small to obtain sufficient kidney tissue.

On the Sheyenne River, over 200 kidney samples were screened for *R. salmoninarum* with the ELISA (Table 6). Antigen was detected in all species collected on the Sheyenne River although four species collected at the downstream site were negative. All fish with detectable antigen were in the low level category except a single green sunfish which had a medium OD reading. Corroborative PCR testing of 23 samples with positive ELISA readings failed to detect

Table 5.— Levels of *R. salmoninarum* determined with an ELISA and corroborative testing with a nested PCR assay for three species of fish collected from Devils Lake. Abbreviated common names for fish are explained in Table 3.

Fish species	ELISA			PCR Assay	
	Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
NOP	41	43.9	Low	3	0
WAE	53	18.7	Low	3	0
YEP	5	20.0	Low	1	0

Table 6.— Levels of *R. salmoninarum* determined with an ELISA and corroborative testing with a nested PCR assay for nine species of fish collected from two sites on the Sheyenne River. Abbreviated common names for fish are explained in Table 3. N/T = not tested.

Fish species	Relative sample site	ELISA			PCR Assay	
		Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
BLB	Up stream	60	91.7	Low	3	0
	Down stream	60	88.3	Low	3	0
CAP	Down stream	1	100.0	Low	1	0
GSF	Down stream	1	100.0	Medium	1	0
NOP	Up stream	7	28.6	Low	2	0
	Down stream	3	0.0	Not detected	0	N/T
SMB	Down stream	1	0.0	Not detected	0	N/T
TMP	Up stream	13	15.4	Low	2	0
	Down stream	3	100.0	Low	3	0
WAE	Up stream	15	33.3	Low	3	0
	Down stream	12	0.0	Not detected	0	N/T
WHS	Up stream	20	95.0	Low	3	0
	Down stream	2	0.0	Not detected	0	N/T
YEP	Down stream	4	50.0	Low	2	0

DNA of *R. salmoninarum*. Results of *R. salmoninarum* screening with samples from the Red River show a similar pattern to Devils Lake and the Sheyenne River. A total of 83 kidney samples were tested and all species were represented with positive ELISA readings (Table 7). The mean antigen level category was low for all species except common carp which had medium antigen level. Corroborative PCR testing of 16 samples with positive ELISA readings failed to detect DNA of *R. salmoninarum*. As with Devils Lake, none of the fish from the Red and Sheyenne rivers had any external or internal clinical signs indicative of BKD or other diseases.

Table 7.— Levels of *R. salmoninarum* determined with an ELISA and corroborative testing with a nested PCR assay for eight species of fish collected from two sample sites on the Red River. Abbreviated common names for fish are explained in Tables 3 and 4.

Fish species	Relative sample site	ELISA			PCR Assay	
		Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
BLB	Down stream	2	100.0	Low	2	0
CAP	Up stream	2	100.0	Medium	2	0
	Down stream	4	100.0	Low	3	0
CCF	Up stream	60	83.3	Low	3	0
	Down stream	9	11.1	Low	1	0
DRM	Up stream	2	50.0	Low	1	0
MOO	Down stream	1	100.0	Low	1	0
NOP	Down stream	1	100.0	Low	1	0
SHR	Up stream	1	100.0	Low	1	0
WHS	Up stream	1	100.0	Low	1	0

**Viral pathogens.**— A total of 132 samples were collected from across the survey area and tested for listed viruses. No viral fish pathogens were detected with the variety of standardized cell culture assays used during the survey.

**Parasitic pathogens.**— *M. cerebralis*, the myxosporean responsible for whirling disease, was the only parasitic fish pathogen included for testing in this survey. A total of 21 samples (5 pooled fish/sample) were collected and processed from across the survey area. At Devils Lake, 2 yellow perch samples were tested and found to be negative. On the Red River, 1 sample of black bullhead and 7 channel catfish samples also tested negative. On the Sheyenne River, we tested 6 samples from black bullhead and 3 from tadpole madtom and all were negative for the parasite.

We observed a small number of spores with morphology similar to *M. cerebralis* from 2 pooled samples of white sucker from the Sheyenne River. DNA from the suspect spores was extracted and tested with the *M. cerebralis* PCR assay. The PCR confirmed that suspect spores were not *M. cerebralis*.

A relatively small number of macroparasites were observed during gross examination of fish. Identification of parasitic worms was beyond the scope of this survey however we provide a general description here. Several surveys for fish parasites have been completed in and around the current survey area including those by Forstie and Holloway (1984), Reinisch (1981), and Sutherland and Holloway (1979). We observed a nematode (round worm) infestation in black bullheads collected from both sampling sites on the Sheyenne River. The worms were encysted along the mesentery mostly associated along the lower gastrointestinal tract. Also, a small number of nematodes were seen in the mesentery of a single walleye collected in the Sheyenne River near Valley City. A nematode similar in appearance was observed in the mesentery of two walleye from Devils Lake. On the Red River, we observed leeches attached to three channel catfish. Judged solely by general fish condition, these parasites did not appear to have a significant impact on either fish growth or survival.

**Fungal pathogens.**— We observed one walleye, collected from the upstream sample site on the Sheyenne River, with numerous lesions on surface of the liver and spleen. The lesions were a creamy white color and circular but did not appear raised. The lesions ranged in diameter from about 1 to 5 mm. Affected tissues were preserved in Davidson's solution, processed with standard histological techniques, stained with either hematoxylin and eosin or Giemsa, and viewed with light microscopy. Microscopic examination showed the walleye was affected by a systemic fungal infection. Numerous fungi were observed in blood vessels and throughout liver and spleen tissues.

## Discussion

During October 2001 and August 2002, we examined and tested more than 500 fish for a list of specific fish pathogens using protocols for the National Wild Fish Health Survey. Overall, the health and condition of fish from Devils Lake and from the Red and Sheyenne rivers appeared to be very good. None of the fish examined, regardless of species, size, or sample site had any external or internal clinical signs that would indicate infection by a fish pathogen included in this survey.

Detection of soluble antigen of *R. salmoninarum* was the only evidence of possible exposure to specific fish pathogens. However, because active infection by *R. salmoninarum* could not be confirmed with the PCR assay, there is reason to believe the ELISA data may represent false positive readings. One explanation for this centers on the nature of the negative reference tissue used to establish the negative-positive threshold for antigen detection. During protocol development for the NWFHS, we recognized the need for a standard ELISA reference tissue so that each of the FWS Fish Health Centers would calculate and report ELISA results consistently.

The reference tissue in current use is pooled kidney from fall chinook salmon which has been verified to be free from *R. salmoninarum*. This kidney generates ELISA OD values from 0.074 to 0.090 which is considered a small amount of variation. It is possible that certain proteinaceous elements or other constituents of non-salmonid kidney interfere with the ELISA and result in higher background readings. It may also be possible that other unidentified bacteria produce a protein similar enough to the p57 antigen of *R. salmoninarum* to cause a cross reaction. Some have argued that negative reference tissue (kidney) should be established for each family of fish intended for screening by the *R. salmoninarum* ELISA.

*R. salmoninarum* was once thought to infect primarily members of the family Salmonidae however sampling efforts of the NWFHS have resulted in detection of the organism in many non-salmonid fishes before this study. The bacterium has been isolated in fish from cold, cool, and warm-water environments. *R. salmoninarum* has been confirmed with the nested PCR assay in at least 32 non-salmonid species comprising eight families including Amiidae, Catostomidae, Centrarchidae, Clupeidae, Cottidae, Cyprinidae, Esocidae, and Ictaluridae. One explanation for these findings is that in many of the cases there exists sympatric salmonid populations. In the present survey, no salmonids were collected in the traps and nets. While we have no previous NWFHS information from North Dakota, in Montana *R. salmoninarum* has been confirmed in mottled sculpin from Reese Creek, Utah chub from Hebgen Lake, shorthead redhorse sucker from Ft. Peck Lake, and in most salmonid species. Antigen of *R. salmoninarum* was detected by ELISA in white sucker from Bear Paw and Beaver Creek reservoirs and in white sucker from Ft. Peck Lake although those infections could not be confirmed with the PCR assay (Peters 2000 and Peters 2001). *R. salmoninarum* was also confirmed in a survey of six lakes in western Glacier National Park, Montana and the estimated prevalence was higher in non-salmonids compared to trout and whitefish (Peters 2002). We have not observed any clinical signs of BKD in non-salmonid fishes in Montana despite a wide range of positive ELISA values and DNA confirmation by PCR.

Prior to the NWFHS, little study was directed at epizootiology of *R. salmoninarum* outside widely recognized Pacific salmon and trout hosts. In a review of host range, Fryer and Lannan (1993) cite successful experimental transmission of *R. salmoninarum* to Pacific herring *Clupea harengus pallosi* (Traxler and Bell 1988) and to sablefish *Anoplopoma fimbria* (Bell et al. 1990), however efforts to infect lamprey *Lampera tridentata* (Bell and Traxler 1986) and common carp *Cyprinus carpio* (Sakai et al. 1989) were not successful. Sampling in cooperation with the NWFHS resulted in detection of *R. salmoninarum* antigen (ELISA) and DNA (PCR assay) in common carp from the Columbia River, Washington; Lake Mohave, Nevada; Mississippi River, Minnesota and Wisconsin; and the Snake River, Idaho and Washington. In Japan, antigen of *R. salmoninarum* was detected with immunoassays in greenling *Hexagrammos otakii*, Japanese sculpin *Cottus japonicus*, and in scallops *Patinopecten yessoensis* collected in relative close proximity to marine netpens holding coho salmon infected with the pathogen (Sakai and Kobayashi 1992). Results of the NWFHS demonstrate that the distribution of *R. salmoninarum* is more wide spread than previously believed. Clearly, further work is needed to better understand host-pathogen interactions that exist between *R. salmoninarum* and non-salmonid fishes in natural environments.

Spores of myxosporean parasites, other than *M. cerebralis*, are frequently observed in extracts from the enzymatic digestion process. Some of these spores have morphology similar to *M. cerebralis* although many are distinctly different in either size, shape or internal characteristics. Until the recent advent of a highly specific PCR assay (Andree et al. 1998), most pathologists used standard histology procedures to confirm the identity of suspect spores observed with screening methods. Confirmation of *M. cerebralis* by histology required the observation of morphologically consistent spores in cartilage and bone. The process can be time consuming and may require several step-sections to isolate and identify infected tissues especially where infection is of low intensity. During this survey, myxospores were observed in the cranial extracts from white sucker collected from the Sheyenne River. Spore morphology was similar enough to *M. cerebralis* to warrant further testing with PCR. It was not surprising that results of the PCR assay were negative. Even though whirling disease is limited to salmonids, we frequently test non-salmonid samples when suspect spores are observed during routine screening. No spores were observed in any other fish from any of the other sample sites.

Although no fish pathogens were detected in this survey, there is reason to view these results with caution. It is possible that a pathogen may have been present in a population but it was not detected because either sample size, population size or presumed prevalence did not fit the sampling model. First, the target sample size of 60 fish of an individual species was collected at some sample sites but in many cases fewer fish were obtained. The target sample size used in this study was based on a 95% confidence level of detecting one infected fish given a presumed pathogen prevalence of 5% and a population size (N) of at least 2,000 individuals (Ossiander and Wedemeyer 1973). In large field surveys such as this, the investigator must speculate on model parameters because information on population size and actual pathogen prevalence are usually unknown. Smaller samples sizes (n) will increase the bound on the error of estimation of the prevalence. In this survey, the population size (N) of certain species may have been less than 2,000 at the time of sampling particular sites. In addition, the actual prevalence of a certain pathogen, if present, may have been lower than 5% and thus require a substantial increase in sample size (n) for pathogen detection. For example, if model parameters were as previously stated but the actual pathogen prevalence was 2%, then the sample size (n) required to detect one infected fish would increase from 60 to 280. Add to this the fact that some fish pathogens experience season changes in occurrence and prevalence. Finally, for some assays, samples were pooled to either increase the amount of tissue for assay (ELISA) or to process large volumes of tissue more efficiently (enzymatic digest and virology). Reporting prevalence of infection based on pooled fish samples may be biased because contribution of individuals within the pool can not be determined and these levels will almost certainly vary. The amount of variation will depend on several parameters not limited to actual pathogen prevalence, if any, and the specificity and sensitivity of the assay used. The Ossiander and Wedemeyer (1973) sampling model assumes that assays have a sensitivity of 100%. As with nearly any assay, there is always some level of risk for false negative results.

### **Recommendations for Future Work**

The present survey was based on samples collected at one point in time. Given the occurrence and prevalence of certain fish pathogens may be variably affected by several life history characteristics and elements of environment, especially those causing increased stress, future surveys should consider sample collections at two or more times during the year. It may be particularly important and interesting to examine fish either during or immediately following spawning activities.

In large field surveys, it is often impractical or even impossible to examine and test every fish for every pathogen or parasite that may be suspect. It is necessary for tissue samples, once collected, to be handled properly and appropriate assays begun within certain time limits. These considerations often preclude the number of fish that can be examined and the number of assays used in any given time frame. For these reasons, specific fish pathogens of importance should be indicated and methods for detection identified before field collections begin. Future surveys should include a thorough review of the pathogen list by basin co-managers

While an adequate sample size was obtained for some species at some of the samples sites, in many cases too few fish of other species were examined to establish the presence or absence of fish pathogens with an appropriate level of confidence. Obtaining sufficient sample size can be a common problem with the initial undertaking of large field studies such as this. It can also be problematic when certain species are of low abundance. Often times, prior information regarding species abundance and distribution are limited. Also, upon initiation of such surveys, it is often difficult to know the type(s) of fishing gear to employ that permit collection of multiple species and maximize catch per unit effort. These elements are made particularly difficult during rapidly changing environmental conditions. Future fish pathogen surveys should identify and focus on species of greatest interest or importance. When appropriate, selection of species should also be related to the particular fish pathogens of concern.

In the present survey, fish in Devils Lake were collected from one site (Six Mile Bay). Future work should consider the merits of sampling additional areas of the lake. Sampling efforts should be coordinated with area fisheries biologists to identify key spawning and rearing habitat.

### **National Wild Fish Health Survey**

The U. S. Fish and Wildlife Service - National Wild Fish Health Survey is a cooperative program designed to gain a better understanding of the distribution of important fish pathogens in free-ranging populations and to make the information readily accessible to stakeholders. More information regarding the National Wild Fish Health Survey can be found at the program website. Details of assay results for this survey can be queried from the National Wild Fish Health Survey database link. <http://wildfishsurvey.fws.gov>

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